

Ultrastructural studies of abaxial and adaxial sides of *in vivo* and *in vitro* leaves in Sainfoin (*Onobrychis viciifolia*)

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Abstract Summary: Leaf specimens of both types of *in vitro* and *in vivo* growth were evaluated in sainfoin (*Onobrychis viciifolia*). It was found that different resistance strategies of Sainfoin can be assessed based on stomata, epicuticular waxes, convex cell, trichomes and calcium oxalate crystals function.

Introduction: Despite the fact that Sainfoin (*Onobrychis viciifolia*) is an important forage crop, it has received little attention and assessment for *in vitro* studies. Wide varieties of plant micro-structures including light reflection and water absorption structures have been already defined by the Scanning Electron Microscope (SEM). Physiological activity of land plants is mainly depends on conservation of water which is carried out via plant roots. In order to hold the water and avoiding of leaching the ions from interior structure in plants, a protective waxy layer called cuticle is developed that covers the epidermis cells from inside the plant [1, 2]. Raising or drop of water loss might be influenced by trichomes function as well as affecting on surface wettability [3].

Materials and methods

Explant Source: Seeds of *Onobrychis viciifolia* existing at gene bank of natural resources, Iran were selected. For *in vitro* growth condition, almost 100% of seeds germinated in the Murashige and Skoog medium (MS) supplemented with 3% (w/v) sucrose and 0.75% (w/v) agar after 1-2 weeks. The seeds and containers were not sterilized in *in vivo* growth culture. The samples were germinated on soiled vase in greenhouse. These samples were also kept in growth room at 25 ± 2 °C, with 70% humidity and 16-h photoperiod.

Scanning Electron Microscopy (SEM): Leaf specimens of both types of *in vitro* and *in vivo* growth were treated with the method of Taha et al., [4]. Eventually, the samples were coated with gold for 1 min, before the observation by SEM (JEOL 6400).

The epidermal peel was done to study the presence of stomata and trichomes on the abaxial and adaxial surfaces, anticlinal walls, types of stomata, epicuticular waxes, convex cells, trichomes, calcium oxalate crystals function and stomata index of the *in vivo* and *in vitro* leaves.

Results: Two different leaf samples of the *in vitro* and *in vivo* (intact plant) have been studied thoroughly. Subsequently, properties of both lower leaf side (abaxial) and upper leaf side (adaxial) were assessed. The course of the anticlinal walls was exposed and polygonal in the abaxial side of the intact and *in vitro* leaves (Figs. 1a and 2a). Jog trichomes non-secreting glands covered by spines (botanically thorns) were observed only in lower leaf side (Figs. 1d and 2d). A convex cell form with irregular cuticular folding in the central fields and parallel folding in the anticlinal field of the cells was observed in the adaxial leaf side of intact plants (Figs. 4a and b). Wax platelets on adaxial sides of the leaves were more than the lower leaf side in both growth cultures (Figs. 1c and 2c). Stomata interrupt the cuticular layer, but can be closed (intact plants) if water becomes unavailable, e.g. when high day temperatures increase the water loss also increase (Figs. 2b and 4d). However, this barrier also limits the uptake of carbon dioxide from the atmosphere for photosynthesis.

Discussion: In the current research, deficit of water storage in black soil which is required for further photosynthesis process is inevitably compensated via the function of *in vivo* leaves. To explore more clarifications, diverse tension-resistance techniques using *Onobrychis viciifolia* were evaluated in the leaves of intact plants when subjected against

water deficiency. Based on a comparison of *in vivo* and *in vitro* leaves, cell shrinking induced by water loss of the cells due to convex cell morphology and sufficient water of MS media in *in vitro* specimens (Fig. 3b). Thus, convex cells are seldom found in water containing epidermal cells when water is scarce in soil (Fig. 4a). Water evaporation rate is controlled by opening (Fig. 3d) and closing (Fig. 4c) of stomata. Closing of stomata takes place in order to reduce the water evaporation when plants can receive insufficient amount of water through the roots. Adversely, stomata are opened when the gas exchange process is the main objective.

Acknowledgements

The authors would like to thank the University of Malaya, Malaysia for the facilities and financial support provided (IPPP Grant PV025/2011B and UMRG grant RP025-2012A).

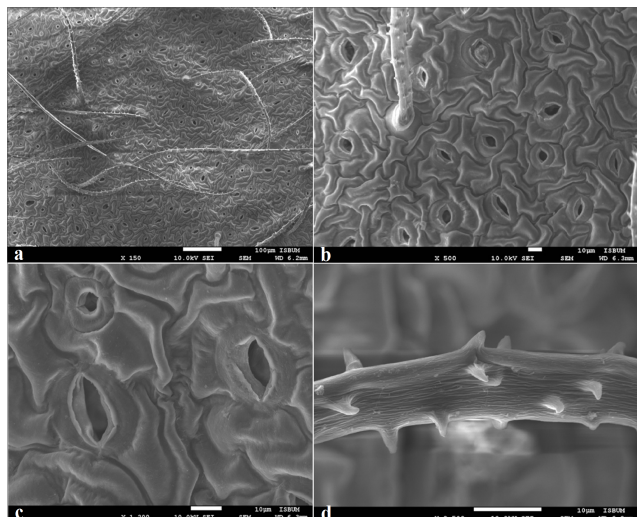


Fig. 1. Abaxial side of *in vitro* leaf

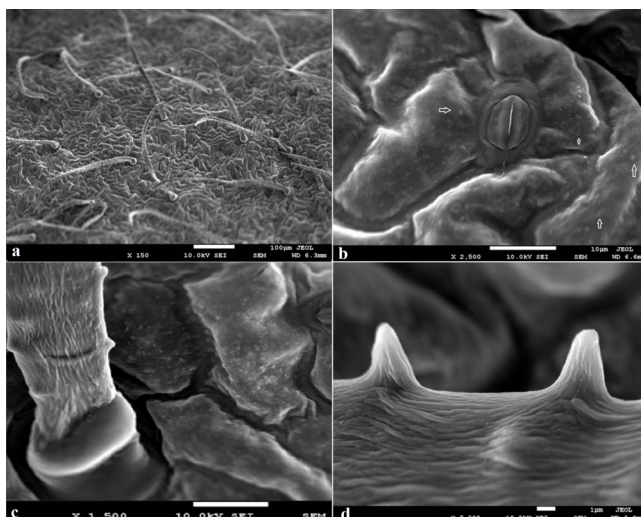


Fig. 2. Abaxial side of *in vivo* leaf

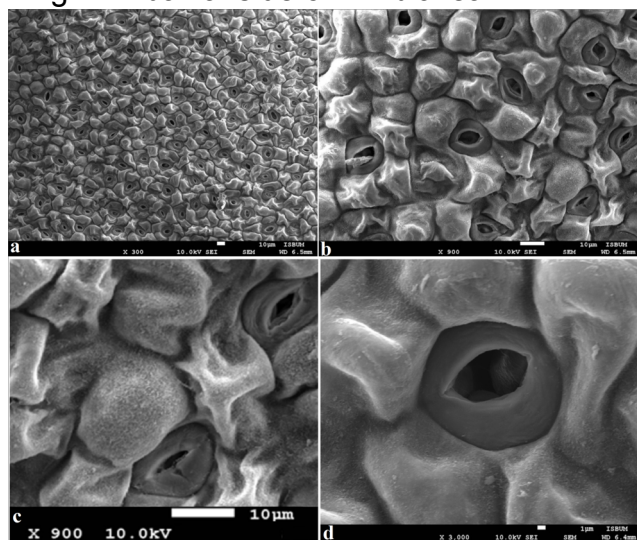


Fig. 3. Adaxial side of *in vitro* leaf

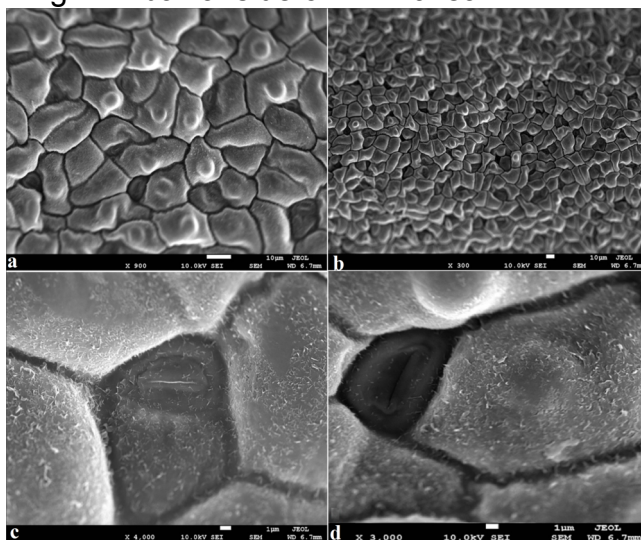


Fig. 4. Adaxial side of *in vivo* leaf

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